EXHIBIT 16

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Virus Neutralization and Virus-Induced Immune Complex Disease

Virus-Antibody Union Resulting in Immunoprotection or Immunologic Injury — Two Sides of the Same Coin[1]

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I. Introduction

Protection against many viral infections is provided by specific antiviral antibody in the serum and other tissue fluids. The existence of this protection has been amply documented by epidemiologic surveys, experimental animal studies, experience with the passive transfer of hyperimmune sera and the use of viral vaccines in man. The mechanism of this protection is of great interest to immunologists and virologists and is usually studied by virus neutralization experiments, in which the decreasing infectious titer of a viral preparation following exposure to heated hyperimmune or convalescent sera is examined [LENNETTE, 1964]. However, these virus neutralization determinations usually fail to consider (1) the class or subclass of antibodies produced during primary, secondary, or persisting infection, (2) varying abilities of these antibodies to bind to and alter virus, and (3) differing abilities of virus-antiviral antibody (V-Ab) union to interact with serum effector systems of which complement is the major one. Such information is necessary to our understanding of the antiviral antibody protective mechanism.

In addition, the neutralization technique gives little information on what occurs after V-Ab union *in vivo*. Complement could enhance virus neutralization by covering the V-Ab complex by simple piling-up of components on the surface of the virion, by enhancing agglutination of V-Ab complex, by altering the surface of the V-Ab complex so that it is handled differently by cell receptors, or by directly lysing the V-Ab mixture. Early in infection antibody may be largely ineffective unless aided by the complement effector system. For example, with primary infection or immunization, most antigens elicit early IgM antibody responses which are soon followed by IgG and IgA responses. When the primary antigenic stimulation is in the respiratory tract or gut, greater amounts of IgA antibodies are made. Following reinfection (secondary response) the antibodies made are predominantly of the IgG class, with some antibodies to IgM and IgA. With continuous antigenic challenge, as seen in persistent virus infections, it appears that IgG is also the predominant class made, although this phenomenon has not been carefully studied. In early primary infection, the IgM antibodies usually are of lower affinity for virus antigens than are the IgG antibodies from secondary or late primary infections; however, IgM antibodies show an enhanced agglutinating ability and greater ability to activate complement when compared to IgG antibodies. Further, IgG subclasses activate complement in varying degrees, i.e., IgG3>IgG1>IgG2, and IgG4 do not activate complement at all [reviewed by SPIEGELBERG, 1974]. While it is not clear

whether IgG can activate complement by the alternate pathway in addition to its activation by the classical pathway, it is clear that IgM activates complement via the classical pathway (see Sect. II. B. 1). IgA activates complement only by the alternate pathway.

Information concerning V-Ab-complement (V-Ab-C) interaction is also important in the study of the role of V-Ab complexes in the pathogenesis of immune complex disease; antigen-antibody complexes may cause significant disease by being trapped in various tissues of the host. In fact, V-Ab immune complex disease has been found to occur frequently in chronic virus infections [OLDSTONE and DIXON, 1971c, 1973] and may be the most common pathogenic mechanism of human nephritides and arteritides.

Virus infection offers many opportunities for the development of immune complex disease. The virus is a self-replicating agent which provides a supply of macromolecular antigens and in most instances elicits a host immune response. In acute viral infection an imbalance of viral replication and immune response leads to either termination of disease or death of the host, while in chronic virus infections the time scale is lengthened and both ongoing virus replication and a continuous host immune response against the virus occur.

The purpose of this review is to summarize some of the work done recently on the characterization of mechanisms by which immune reactions neutralize virus and/or play a role in the pathogenesis of tissue injury in virus infections. No attempt is made to review all the scientific literature published on these topics; rather, I will emphasize recent advances in this field which either establish new concepts or serve to alter old ones. For reviews on the formation and dissociation of V-Ab complexes and the process of neutralization or immune complex disease in general, the reader is referred to the following: DULBECCO *et al.* [1956]; FAZEKAS DE ST. GROTH [1962]; FENNER [1968]; SVEHAG [1968] (virus neutralization); and COCHRANE and KOFFLER [1973] (immune complex disease).

II. Virus Neutralization by Antibody

A. Introduction

Neutralization of virus is measured by determining the decrease in infectious titer of a viral preparation following exposure to antibody. Treatment with antibody does not usually cause the irreversible loss of infectivity

because simple techniques such as dilution or any one of a number of physical and chemical treatments restore virus infectivity [DULBECCO *et al.*, 1956; HUMMELER and KETLER, 1958; FAZEKAS DE ST. GROTH, 1962; LAFFERTY, 1963; GRANOFF, 1965; FENNER, 1968; SVEHAG, 1968].

Antibody may neutralize virus infectivity in several ways [DULBECCO *et al.*, 1956; FAZEKAS DE ST. GROTH, 1962; DALES and KAJIOKA, 1964; MANDEL, 1967a, b; FENNER, 1968; SILVERSTEIN, 1970], the way being dependent on the virus-host cell system studied. Best known are the abilities of neutralizing antibody to inhibit the attachment of virions to cultured cells, prevent the engulfment of attached virions, forestall the release of virus nucleic acid and increase the degradation of virus nucleic acid. Antibody can also neutralize virus by covering the virion surface with antibody molecules, by agglutinating with large aggregates of virions and antibody molecules functioning as a single infectious unit, or by sterically hindering the virus-cell receptor site [SVEHAG, 1968; NOTKINS, 1971]. Less well known is the ability of monocytes and fixed phagocytes of the reticuloendothelial system to inactivate V-Ab mixture. According to SILVERSTEIN [1970], macrophages engulf and degrade neutralized poxvirus-antibody complexes to low molecular weight metabolites. The action is probably similar to the action of antibody as an opsonin in bacterial systems. Further, these studies indicate a fundamental difference between acute and chronic virus infections. In acute virus infections, virus *per se* can replicate in and destroy macrophages, but is degraded when attached to antibody; whereas in chronic virus infections [MIMS and SUBRAHAMANYAN, 1966; NOTKINS *et al.*, 1966b; YOBAYASKI and KONO, 1967; PORTER *et al.*, 1969], V-Ab complex is picked up by macrophages, in which the virus is able to persist and replicate. The process probably proceeds through a receptor for Fc (immunoglobulin Fc fragment) on the surface of macrophages. Further, the demonstration of Fc receptor on other cells, including lymphocytes, monocytes and polymorphonuclear leukocytes suggests that such receptors may be part of a mechanism for attaching viruses to these particular cells.

B. Participation of Complement

1. Components of Complement

In the last few years there has been a series of papers from various laboratories reporting on the effects of serum accessory or heat labile serum factors on virus neutralization [LENNETTE, 1964; SVEHAG, 1968; WAY and GAR-

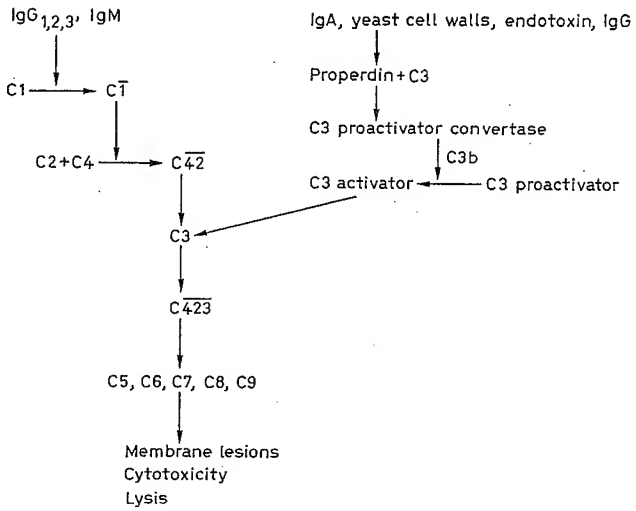

Fig. 1. Schematic drawing of the two mechanisms of complement activation.

WES, 1970; CHAPPELL *et al.*, 1971; YOSHINO and KISHIE, 1973]. These reports indicate the participation of complement in *in vivo* viral neutralization.

Complement consists of a number of immunochemically distinct protein components normally present in the circulation and other tissue fluids in inactive precursor forms. After contact with antigen-antibody complex, these components are activated sequentially and are associated with biological actions such as immune adherence, opsonization, release of anaphylatoxins, vasoactive amines and chemotaxic factors and with lesions on plasma membranes leading to lysis [reviewed by MÜLLER-EBERHARD, 1968, 1972; COOPER, 1972a, b].

C1, the first complement component, consists of three different types of subunit: C1q, C1r, and C1s. C1 reacts with the antibody portion of the immune complex through the C1q subunit. There follows a series of autocatalytic reactions which result in assembly of the various complement components. As shown in figure 1, C2 and C4 are the inactive precursors of the enzyme $C\overline{42}$ which is necessary for action on C3. After the reaction with C3, this enzyme becomes $C\overline{423}$. Subsequently, C5, C6 and C7 react and then C8 and C9, the terminal components, bind as a complex on the attacked membrane. Recently, the existence of a second recognition and activation mechanism

in serum which can bypass C1, C2 and C4 has been shown (fig. 1) [SANDBERG *et al.*, 1970; GÖTZE and MÜLLER-EBERHARD, 1971]. The alternate pathway, also known as the properdin system, consists of a number of serum proteins of which four have been identified: properdin, C3 proactivator, C3 proactivator convertase and C3. Heating of serum at 50 °C for 20 min specifically destroys C3 proactivator.

To study the interaction of V-Ab complex with complement, the various purified complement components can be added in sequence. Other more accessible reagents are serum heated at 56 °C for 30 min to study the classical complement system, serum heated at 50 °C for 20 min to study the alternate complement pathway, and various sera deficient in unique complement components, e.g., C2-deficient human serum, C4-deficient guinea pig serum, C5-deficient mouse serum and C6-deficient rabbit serum.

2. Covering of Virus with Antibody and Complement

The ability of complement to enhance neutralization of V-Ab complex by a blanketing effect was first reported by BERRY and ALMEIDA [1968]. Utilizing electron microscopy and neutralization tests, these investigators noted that the addition of heated hyperimmune fowl antibody to avian infectious bronchitis virus caused large aggregates of virus particles and a halo surrounding the virus which measured 30 nm. When unheated fowl antisera or heated fowl antisera with a complement source were added to the virus preparation, no overall change in virus aggregation occurred, but the virus halo now measured up to 70 nm. There was no evidence of any other complement-mediated alteration, e.g. viral lysis (see below, sect. II. B. 4). Further, the presence of complement resulted in the inactivation of 99% (2.5 logs) more virus than with antisera alone. This suggested that the role of complement in neutralization in this system was probably that of contributing bulk in the form of large protein molecules to V-Ab complex.

Further evidence that complement could produce neutralization by the piling-up of components on the surface of the virion was demonstrated in experiments using functionally pure complement components [DANIELS *et al.*, 1970]. In these experiments herpes simplex virus complexed with immunoglobulin M was neutralized by serum deficient in the fifth and sixth components of complement but not by serum deficient in the fourth component of complement. Further, the addition of an optimal amount of purified C1 and a high concentration of C4 also resulted in neutralization. With less C4, less virus was neutralized until either C4 concentration was increased or C2 and C3 were added to the V-Ab-$C\overline{14}$ complex. As C1 and C4 alone

are not known to cause aggregation of antigen-antibody complexes, the experiments using only C1 and C4 show that enhanced virus neutralization occurred in the absence of any additional aggregation. To further demonstrate this point, NOTKINS and co-workers [NOTKINS, 1971; NOTKINS *et al.*, 1971] mixed radiolabeled herpesvirus with antibody or with antibody and complement and measured the sedimentation of virus, and V-Ab or V-Ab-C complexes by rate-zonal centrifugation. Increasing concentrations of antiviral antibody shifted the peak of radioactivity to the bottom of the tube, indicating enhanced cross-linkage of V-Ab complexes with higher antibody concentrations. When fresh serum (C) was added to the V-Ab complex, no increase in the density of V-Ab-C complex was noted over the density of V-Ab complex alone.

All the above experiments indicate that this particular neutralizing effect of complement (1) does not require the terminal complement components (C5 through C9), and (2) produces neutralization by piling up the early complement components on the surface of the virion. Thus, complement appears to neutralize virus by contributing bulk around the V-Ab complex in the form of large protein molecules.

In addition to contributing bulk, the presence of complement components may lead to distortion of virion membrane due to hydrophobic interactions and other binding changes. Such membrane distortion and swelling have been seen on the surface of sensitized red blood cells following the binding of late-reacting complement components [POLLEY, 1971].

3. Agglutination of Virus by Antibody and Complement

Another way in which complement can neutralize virus is by enhancing aggregation of V-Ab complexes [LINSCOTT and LEVINSON, 1969; WALLIS and MELNICK, 1971; OLDSTONE *et al.*, 1972a]. In experiments studying herpesvirus neutralization, WALLIS and MELNICK [1971] showed that anti-herpesvirus antibody collected from rabbits 8 days after immunization with virus was able to form V-Ab complexes. However, significant virus neutralization did not occur unless the same antisera were used unheated (antibody and complement source). These authors interpreted their experiments as showing that upon the addition of complement large aggregates containing many virions and antibody molecules were formed which then functioned as a single infectious unit. An alternate interpretation of the WALLIS and MELNICK experiment is that offered by NOTKINS and co-workers [DANIELS *et al.*, 1970; NOTKINS, 1971; NOTKINS *et al.*, 1971], discussed in section II. B. 2, which suggests that neutralization of herpesvirus by early IgM antibody and

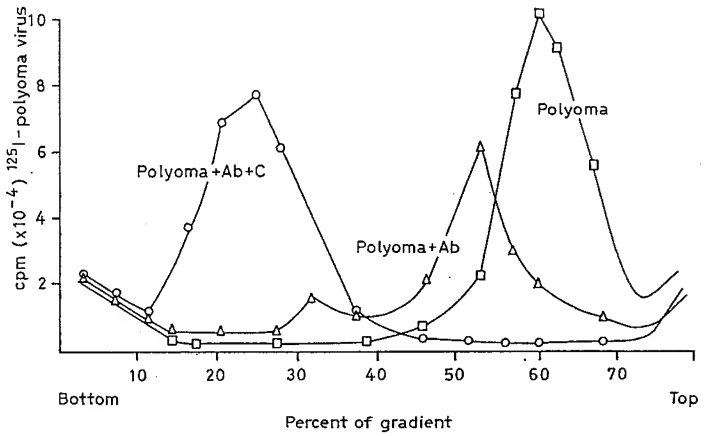

Fig. 2. ^{125}I-polyoma virus was incubated with antibody and complement and then placed on 5–20% sucrose density gradient for rate-zonal centrifugation analysis. Using phage markers, the s rate of the V-Ab complex was 270s while the V-Ab-C complex was 450s [OLDSTONE, COOPER and LARSON, J. exp. Med. *140:* 549, 1974].

complement is due to piling up of the early complement components on the surface of the virion. Further density gradient studies using early and late complement components as well as electron micrographic study of V-Ab-$\overline{C1423}$ and V-Ab-C complex should clarify this issue.

In our laboratory [OLDSTONE *et al.*, 1972a], neutralization of polyoma virus-Ab complex was enhanced with the addition of purified first four components of complement (C1, C4, C2, C3), while C1 and C4 in excess or C1, C4, C2 had no neutralizing effect. Similar results were reported by LINSCOTT and LEVINSON [1969], who noted that early IgM antibodies neutralized or enhanced the neutralization of Newcastle disease virus in the presence of purified C1, C4, C2 and C3.

To understand better the interaction among virus, antibody and complement, we have also purified and radiolabeled different viruses and studied their interaction with antibody, complement and various isolated complement components. In this type of experiment, the outer coat of the virus was labeled with ^{125}I [LARSON and OLDSTONE, manuscript in preparation] or the viral nucleic acid was labeled with ^{14}C or ^{3}H. Radiolabeled virus was incubated with limiting amounts of antibody and complement or complement components, placed on 5–20% sucrose gradients and analyzed by rate-zonal centrifugation. The results with polyoma virus are shown in figures 2–4. In

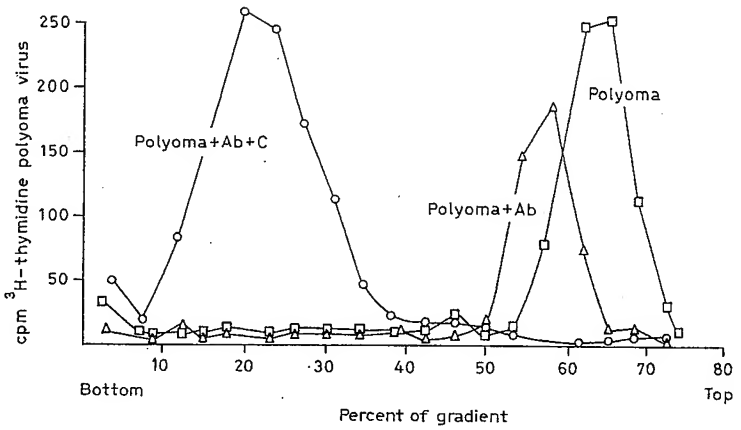

Fig. 3. ³H-thymidine-labeled polyoma virus was incubated with antibody and complement and then placed on a 5–20% sucrose density gradient for rate-zonal centrifugation analysis. Note the similarities between figures 2 and 3. The sizes of the various complexes were 270s and 450s for V-Ab and V-Ab-C, respectively. ³H-thymidine DNA was not susceptible to deoxyribonuclease digestion. Figures 2 and 3 represent enhanced aggregation of V-Ab complex with the addition of complement. There is no evidence of lysis of virus. Further electron microscopic study of V-Ab-C complex showed marked enhancement of clumping and covering of polyoma virus particles over that seen with V-Ab alone. No pits or craters were seen on the virus membrane (compare with fig. 5 and 6) [OLDSTONE, COOPER and LARSEN, J. exp. Med. *140:* 549, 1974].

earlier experiments we noted that the addition of increasing amounts of antibody enhanced the viral sedimentation rate in increments, suggesting cross-linking of viral particles. The use of either fresh serum, isolated C1q or C1, C4, C2, C3, but not C1, C4, or C2, significantly enhanced V-Ab cross-linking (fig. 2–4), suggesting the presence of receptors for complement components on the V-Ab-C complex. Quantitation of individual complement components after the addition of polyoma V-Ab complex to fresh serum indicated that both the classical and alternate pathways of complement activation were triggered and all 9 complement components consumed. Viral lysis was not observed (sect. II. B. 4), although the addition of C1, C4, C2, C3 to V-Ab did significantly enhance neutralization. These experiments indicate that complement further aggregates V-Ab complex, that this occurs with the early complement components and that the latter components (C5–C9) are not required.

Sedimentation of ^{125}I-polyoma virus on 5–20% sucrose gradients

Sample	Percent of Gradient			
	0–20 (bottom)	20–40	40–60	60–80 (top)
V				×
V+Ab			×	
V+Ab+C		×		
V+Ab+△C			×	
V+Ab+C1C4			×	
V+Ab+C1C4C3			×	
V+Ab+C1C4C2C3	×			
V+Ab+C1q	×			

Fig. 4. In these experiments, ^{125}I-radiolabeled polyoma virus was incubated with antibody and complement or complement components, placed on a 5–20% sucrose gradient and analyzed by rate-zonal centrifugation. The results show that the use of fresh serum, isolated C1q or C1, C4, C2, C3 – but not C1, C4 or C1, C4, C2 – significantly enhanced V-Ab cross-linking [OLDSTONE *et al.*, 1972a].

The mechanism by means of which C1, C4, C2, C3 clumps V-Ab complex is not yet clear. The experiments using V-Ab-C$\overline{1423}$ suggest that aggregation of V-Ab complex by early complement components may be through a C3 receptor on the virion. Experiments exploring this possibility are now in progress.

The binding of C$\overline{1423}$ to V-Ab complex has additional theoretical implications. Macrophages, monocytes, polymorphonuclear leukocytes and certain lymphocytes have receptors for C3 and can bind complexes bearing C3 on their surfaces (immune adherence). This binding may be followed by phagocytosis of the V-Ab-C$\overline{1423}$ complex. This may in turn result in the selective concentration of virus in certain cells, thus showing itself to be a mechanism by means of which the host rids itself of unwanted agents. On the other hand, this process may result in the virus gaining access to cells in which it can persist. The probable importance of phagocytic cells in both acute and chronic virus infections is also discussed in section II. A.

4. Lysis of Virus by Antibody and Complement

The role played by complement in causing lysis of cells in the presence of antibody (immune cytolysis) has long been recognized. It appears that this attack of complement is directed against the lipid moiety of the cell membrane and requires the participation of later components, C5–C9 [MÜLLER-EBER-

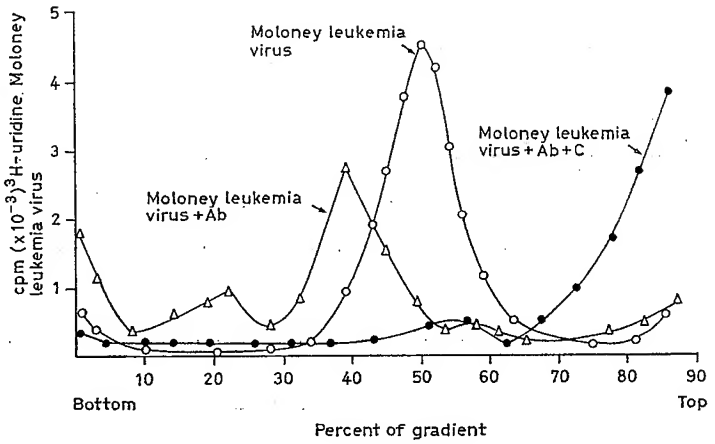

Fig. 5. ^{3}H-uridine-labeled Moloney leukemia virus was incubated with antibody and complement and then placed on 15–20% sucrose density gradient for rate-zonal centrifugation analysis. Note that with the addition of complement to V-Ab complex there was a release of ^{3}H-uridine RNA which is now located near the top of the gradient. This light ^{3}H-uridine RNA was digested by ribonuclease whereas ribonuclease failed to digest the ^{3}H-uridine RNA in the virus or V-Ab preparations (compare with fig. 3).

HARD, 1968, 1972; COOPER, 1972a, b; MAYER, 1972]. Red blood cell membranes lysed with antibody and complement and observed by electron microscopy [HUMPHREY and DOURMASHKIN, 1965] show distinct ultrastructural pits or craters 8–10 nm in diameter.

Using the avian infectious bronchitis virus system described in section II. B. 2, BERRY and ALMEIDA [1968] showed that the addition of unheated rabbit antibody to avian infectious bronchitis virus or heated antiserum mixed with a complement source caused similar pits or lesions of approximately 10 nm in the virus envelope. These and identical observations of the formation of pits of about 10 nm in the virus envelope of both influenza virus and rubella virus have been described, using the term virolysis [ALMEIDA and WATERSON, 1969a], and may well represent a general occurrence among viruses which contain a lipoprotein membrane.

Additional support for the theory of immune virolysis has been provided by OROSZLAN and GILDEN [1970], who showed the release of viral nucleic acid and internal group-specific virus antigen from AKR Gross leukemia virus after the addition of specific antibody to envelope antigens and com-

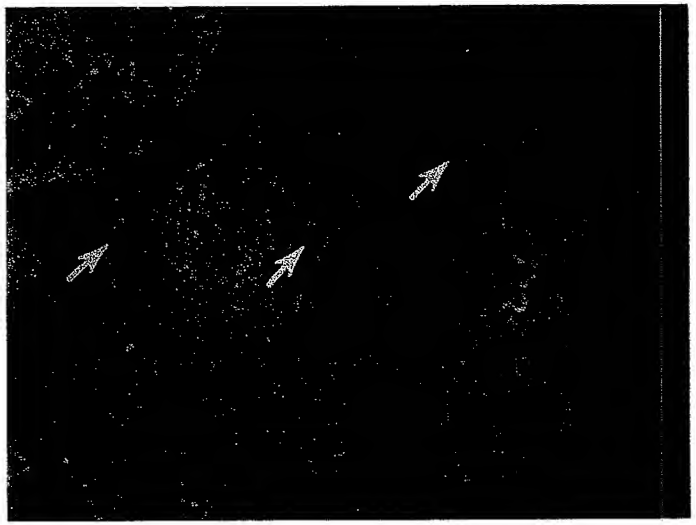

Fig. 6. Pits or craters are found on the surface of Moloney leukemia virus following the addition of antibody and complement. These lesions (arrows) are approximately 10 nm in diameter and resemble those that occur in red blood cell membranes during hemolysis. The addition of antibody alone does not cause lesions.

plement. In this report, the addition of antibody alone to virus was not associated with any release of ^{3}H-uridine RNA, as the labeled portion of virus was resistant to ribonuclease treatment. Upon addition of complement to V-Ab mixtures, the ^{3}H-uridine label was no longer associated with the region of the gradient for the intact virus and was now digested by ribonuclease.

Experiments in our laboratory using ^{125}I or ^{3}H-uridine labeled Moloney leukemia virus on 15–60% sucrose gradient showed that addition of increasing amounts of antibody enhanced the viral sedimentation rate but was not associated with release of ^{3}H-uridine labeled viral RNA, indicating that cross-linking of viral particles but not immune virolysis took place. Electron microscopy of these preparations showed virion aggregation and no evidence of pits or craters in the viral envelope. In contrast, when we added fresh serum to the V-Ab mixture, both the release of labeled viral nucleic acid and pits or craters measuring 10 nm were seen on the virion surface (fig. 5, 6); the free ^{3}H-uridine RNA near the top of the gradient was now susceptible to ribonuclease digestion. Since cell lipoprotein membranes in general are susceptible to the lytic action of antibody and complement, it is not an un-

expected finding that C-type RNA oncornaviruses which mature and bud from the cell surface and acquire a lipoprotein envelope in the process are lysed by antibody and complement.

5. Neutralization of Virus by Complement

Via the alternate pathway the complement system may be activated by aggregates of IgA, perhaps IgG, and by a variety of naturally occurring plant and bacterial polysaccharides [GÖTZE and MÜLLER-EBERHARD, 1971]. Can viruses themselves activate the classical or alternate complement system? Could certain viruses have complement receptors on their surfaces? While the answers to these questions are unknown at present, recent preliminary experiments in our laboratory suggest that viruses themselves may activate complement and in turn be neutralized. Vesicular stomatitis virus was neutralized by fresh serum from many mammals as well as by serum deficient in the fourth, fifth and sixth components of complement. The addition of functionally purified first component of complement (C1) neutralized virus. This experiment using purified C1 suggests that under certain conditions, in the absence of immunoglobulin, complement itself can effectively decrease the titer of infectious virus. While it is not clear how C1 can neutralize virus, the likely possibilities are that it may occur by covering or aggregating the virion.

III. Virus-Induced Immune Complex Disease

A. Introduction

In many virus infections, antibody to virus interacts with virus or viral antigens in the circulation, resulting in the formation of V-Ab complexes. V-Ab complexes themselves are potent pathogenic agents and once deposited in tissues induce a phlogogenic response. V-Ab immune complex disease occurs frequently in chronic viral infections (table I) and may be the most common pathogenic mechanism of animal nephritides and arteritides, exhibiting an immunopathologic picture very similar to chronic human nephritis and arteritis. Further, V-Ab complexes probably occur in most acute virus infections as well.

Much of our insight into the mechanism of immune complex disease has come from the study of animal models of experimental serum sickness. Utilizing these models, DIXON and co-workers have shown soluble antigen-antibody complexes in the circulation and localized antigen and antibody

at the sites of tissue injury and have quantitated the amounts of antigen and antibody bound in the renal glomeruli in both acute and chronic immune complex disease [DIXON *et al.*, 1961; DIXON, 1963; WILSON and DIXON, 1970, 1971]. A major implication of this work is that disease caused by antigen-antibody interaction depends largely upon the *quantitative relationships* of the two reactants. It was also found that chronic disease related to daily injections of low amounts of serum proteins is most likely to occur in poor antibody producers where soluble antigen-antibody complexes are in antigen excess. A similar antigen excess occurs in the majority of chronic viral infections in which a persistent viremia stimulates a continuous low-level immune response with resultant formation of V-Ab complexes [OLDSTONE and DIXON, 1971a]. However, animals that have high antibody responses (in antibody excess) may also develop immune complex disease when given greater amounts of antigen. For example, in Aleutian disease of mink there is a persistent viremia and a continuous high-level immune response with the formation of V-Ab complexes [PORTER *et al.*, 1969]. Whether large antigenic loads occur spontaneously in Aleutian disease and account for the manifestations of immune complex disease is not clear at present.

Most viruses causing chronic immune complex disease are relatively noncytopathic and by themselves cause little tissue injury in the absence of a host antiviral immune response. While many of these viruses share the common properties of containing RNA nucleic acid, budding from plasma membranes, having lipoprotein outer coats, persisting and causing relatively little cytopathology in cultured cells, other viruses contain DNA nucleic acid. These DNA viruses are also associated with persistence and plasma cell membrane alterations and do not usually cause acute cell cytotoxicity in culture. In such situations, the interaction of antiviral antibody with virus and virus-coated membrane antigens probably makes a significant contribution to the pool of circulating immune complexes.

B. Evidence of Immune Complex Disease

Evidence for V-Ab immune complex disease lies in (1) demonstrating circulating V-Ab complexes and (2) showing localization of virus, host immunoglobulin (Ig) and complement at the sites of tissue injury. Several techniques used to show circulating V-Ab immune complexes are C1q agar precipitation, complement utilization, electron microscopy, analytical ultracentrifugation, monoclonal rheumatoid factor precipitation, platelet agglu-

Table I. Virus-induced immune complex disease

Infection	Presence of circulating V-Ab complexes	Granular deposits of host Ig, C3 in tissues	Recovery of specific viral antibody from injured glomerulus	References
Man				
Hepatitis type B	+	+	+	ALMEIDA and WATERSON [1969b]; COMBES *et al.* [1971]; GOCKE *et al.* [1971]; ALMEIDA [1972]; NOWOSLAWSKI *et al.* [1972]; NOWOSLAWSKI [in press]
Burkitt's lymphoma (Epstein-Barr virus)	+	+	+	MAKOJIMA *et al.* [in press]; OLDSTONE and KLEIN [unpublished observations]
Dengue hemorrhagic fever	+	+	unknown	THEOFILOPOULOS and DIXON [personal commun.]
Subacute sclerosing panencephalitis (measles virus)	+	+	+	DAYAN and STOKES [1972]; PHILLIPS [1972]; LAPOINTE *et al.* [1972]; WHITAKER and ENGEL [1972]; OLDSTONE [unpublished observations]
Mouse				
Lactic dehydrogenase	+	+	+	NOTKINS *et al.* [1966]; OLDSTONE and DIXON [1971b]; PORTER and PORTER [1971]
Lymphocytic choriomeningitis	+	+	+	OLDSTONE and DIXON [1967, 1969, 1970b]
Oncornaviruses				
Moloney leukemia	+	+	+	HIRSCH *et al.* [1969]
Murine sarcoma	+	+	+	HIRSCH *et al.* [1969]
Spontaneous Gross leukemia	+	+	+	OLDSTONE *et al.* [1971, 1972]; HOLLIS *et al.* [ms. sub.]; HANNA [personal commun.] ; BARNES [personal commun.]

Table I (continued).

Infection	Presence of circulating V-Ab complexes	Granular deposits of host Ig, C3 in tissues	Recovery of specific viral antibody from injured glomerulus	References
Oncornaviruses, cont.				
Friend leukemia	unknown	+	unknown	OLDSTONE *et al.* [1972]
Rauscher leukemia	unknown	+	unknown	OLDSTONE *et al.* [1972]
Rowson-Parr	unknown	+	unknown	BENDINELLI *et al.* [1953]; MICHAELS *et al.* [1972]
Polyoma	unknown	+	+	TONIETTI *et al.* [1970]
Coxsackievirus B	unknown	+	unknown	SUN *et al.* [1967]
Cytomegalovirus	unknown	+	+	OLDING and OLDSTONE [unpublished observations]
Mink				
Aleutian disease	+	+	+	PORTER and LARSEN [1967]; HENSEN *et al.* [1969]; PORTER *et al.* [1969, 1973]
Horse				
Equine infectious anemia	+	+	+	BANKS and HENSON [1969]; BANKS *et al.* [1972]; MCGUIRE *et al.* [1972]
Pig				
Hog cholera	unknown	+	unknown	CHEVILLE and MENGELING [1969]

Table II. Demonstration of virus-Ab-C complexes in the circulation of mice chronically infected with LDV or LCM virus

Chronic virus infection	Treatment of infectious sera with:	Virus titer
LDV[1]	normal sera	2.8
	antibody to human Ig	2.7
	antibody to mouse Ig	2.0
	antibody to mouse sera	1.4
LCM virus[2]	normal sera	3.5
	antibody to mouse IgG	<1.0
	antibody to mouse IgA	2.9
	antibody to mouse IgM	3.4
	antibody to mouse C3	1.6
	antibody to mouse albumin	3.5

1 Sera from mice infected with LDV was diluted to contain approximately 2.8 ID_{50}/ml and then incubated at 37°C for 1 h with various antisera. The reaction mixtures were then serially diluted and the virus titer, expressed as ID_{50}/ml (log 10), was determined. Reprinted by permission from J. exp. Med. [NOTKINS *et al.*, 1966].
2 Sera from mice infected with LCM virus was mixed and incubated at 37°C for 20 min and 4°C for 2 h. The reaction mixture was centrifuged and the supernatant diluted and inoculated intracerebrally into weanling mice. The virus titer is the reciprocal of log dilution needed to kill 50% of the challenged mice. Reprinted by permission from J. Immunol. [OLDSTONE and DIXON, 1970b].

tination, precipitation of V-Ab complex with antibody directed toward the antibody or complement bound with the virus (anti-Ig or anti-C3 precipitation) and use of cultured cells that contain receptors for bound C3 [NOTKINS *et al.*, 1966a; ALMEIDA and WATERSON, 1969b; OLDSTONE and DIXON, 1969, 1970b, 1971a; KUNKEL, 1971; AGNELLO *et al.*, 1971; NOTKINS, 1971; ALMEIDA, 1972; BOKISCH and THEOFILOPOULOS, 1973]. Among these, the two which appear most promising are the anti-Ig or anti-C3 precipitation and the use of indicator cells containing receptors for bound C3.

That virus travels in the circulation complexed with host Ig was first demonstrated by NOTKINS *et al.* [1966a] who precipitated Ig from the sera of mice chronically infected with lactic dehydrogenase virus (LDV), significantly reducing the serum infectivity titer (table II). Similar results have been obtained in mice chronically infected with lymphocytic choriomeningitis (LCM) virus [OLDSTONE and DIXON, 1970b], Moloney leukemia and murine sarcoma viruses [HIRSCH *et al.*, 1969], mink infected with Aleutian disease

virus [PORTER and LARSEN, 1967], horses infected with equine infectious anemia virus [MCGUIRE *et al.*, 1972] and man infected with hepatitis B antigen (HB Ag) [EDGINGTON, personal commun.]. In addition, OLDSTONE and DIXON [1970b] have shown that virus in the sera of mice chronically infected with LCM virus circulates complexed not only with Ig but also with C3, since immunologic specific precipitation of either Ig or C3 removed at least two logs of infectivity (table II). It was possible to identify the Ig class on the V-Ab complex because precipitation of IgG from the sera removed infectivity, whereas similar precipitation of IgA or IgM did not (table II).

The use of indicator cells containing receptors for C3 shows great promise in determining circulating V-Ab complexes. Recently, BOKISCH and THEOFILOPOULOS [1973] have reported that a continuous human lymphocyte line, Raji, has receptors for the Fc fragment of human Ig and bound C3. Extending these observations, THEOFILOPOULOS blocked the Fc receptor, incubated the cell with preformed protein immune complexes and showed the attachment of complexes to Raji cells. In other experiments Raji cells were mixed with sera from uninfected mice or mice chronically infected with LCM virus. Sera from uninfected mice failed to adhere to Raji cells, but sera from LCM-infected mice bonded to Raji cells, demonstrating the ability of this assay to detect circulating V-Ab complexes [THEOFILOPOULOS *et al.*, 1974].

Deposition of circulating V-Ab complexes in tissues is best determined by identification of virus (viral structural or coded antigens), host Ig and complement in a granular pattern along the basement membrane(s). The immunofluorescent and electron microscopic findings with tissues containing immune complexes are characteristic (fig. 7–9, 11, 13) [DIXON, 1963; COCHRANE and KOFFLER, 1973]. Identification and quantitation of the specific antiviral antibodies present in the deposited complex are accomplished by (1) elution of the glomerular-bound Ig by either low ionic, high ionic or low pH buffers to dissociate V-Ab bonds, (2) recovery of the eluted Ig and its quantitation by radioimmunodiffusion and (3) quantitation of the Ig after absorption with various virus, tissue and cellular antigens, again by radioimmunodiffusion [OLDSTONE and DIXON, 1969, 1971c]. Serum Ig is immunochemically isolated and assayed in a similar way. The ratio of antiviral antibody to total Ig in the tissue eluate over the antiviral antibody to total Ig in the serum depicts the concentration of antiviral antibody localized in the tissue. While such elution studies have certain limitations – most notably loss of eluted antibody via recombination with eluted antigens, incomplete elution or denaturation of eluted antibody – they nevertheless provide the only direct quantitation of antibodies present in the injured tissues.

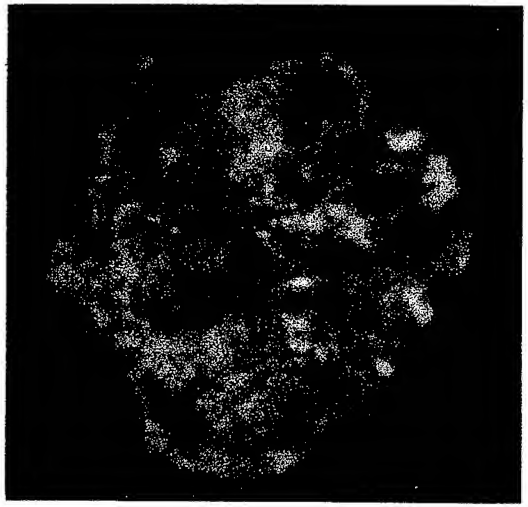

Fig. 7. Fluorescent photomicrograph of a renal glomerulus from an 8-week-old mouse persistently infected with LCM virus. The preparation was stained with a fluorescein conjugated antiserum to mouse IgG. Host IgG is heavily deposited in the mesangial areas and along peripheral capillary walls. Comparable results occurred with fluorescein conjugated antiserum to mouse C3. In contrast, fluorescein conjugated antisera to mouse albumin or fibrinogen did not stain glomerular deposits and matched, uninfected control mice showed no significant Ig deposits. Persistent infection occurs in mice inoculated with LCM virus shortly after birth or infected transplacentally *in utero*. Such chronically infected mice carry high titers of virus in blood and organs and mount an anti-LCM antibody response throughout their lives. These mice develop an associated chronic disease consisting of glomerulonephritis, arteritis, focal hepatic necrosis, extensive lymphoid proliferation, and interstitial round cell infiltrations in many of the body's tissues. The development of disease is related to the amount of LCM virus carried, the size of the anti-LCM antibody response made and the interaction between virus and antibody.

C. Sites of Immune Complex Deposits

1. Renal Glomerulus

Morphologic and immunopathologic study of tissues from animals and man with immune complex disease indicates that the glomeruli in the kidney, the choroid plexus in the brain and, to a lesser extent, blood vessels are the main sites for deposition of circulating immune complexes leading to tissue injury. Of these sites, the renal glomeruli are the most frequently involved

Fig. 8. Electron photomicrograph of a glomerulus taken from an 8-month-old mouse persistently infected with LCM virus. Note lumpy deposition of electron-dense material (D) along the outer aspect of the glomerular basement membrane (GBM). EP, epithelial cell. Reprinted by permission from J. exp. Med. [OLDSTONE and DIXON, 1971a].

and have been studied in most detail. V-Ab complexes accumulate in the glomeruli by entrapment in the glomerular filter and, when their rate of elimination or degradation is slower than that of deposition, complexes gradually accumulate. The histologic appearance of the associated glomerulonephritis varies from early proliferation of endothelial and mesangial cells to later progression of basement membrane thickening, intracapillary hyalinization, and capillary occlusion. Immunofluorescent study of the glomeruli is diagnostic for immune complex deposits and shows accumulation of host Ig, C3 and viral antigen in irregular granular deposits along the capillary walls and in the mesangia (fig. 7–9). Usually, viral antigen is barely visible in the glomeruli unless the covering Ig is eluted prior to fluorescent staining. Electron microscopic study shows lumpy deposits of electron-dense material along the outer aspect of the glomerular basement membrane and in the mesangium (fig. 8). Occasionally, deposits of electron-dense material are seen in the basement membrane and/or along its inner aspect. Visualization of

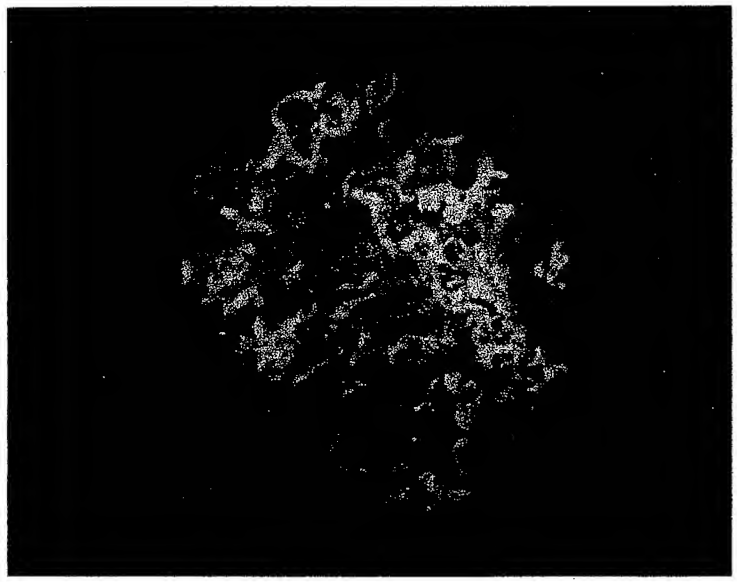

Fig. 9. Fluorescent photomicrograph of a renal glomerulus from an 8-month-old AKR mouse. The preparation was stained with a fluorescein conjugated antiserum to mouse IgG. The IgG is deposited in the mesangial areas and along peripheral capillary walls. The glomerular-bound IgG from similar AKR glomeruli was eluted and shown to contain antibodies to Gross virus, to Gross cell surface antigen and to RNA-dependent DNA polymerase of murine leukemia virus.

viral particles in or along the glomerular basement membrane has been reported [RECHER *et al.*, 1966; KAJIMA and POLLARD, 1970; BATZING and HANNA, 1973].

While circulating V-Ab complexes are commonly found in chronic viral infections, they need not always deposit heavily in tissues and cause disease [OLDSTONE and DIXON, 1971a]. In both chronic LCM virus and LDV infections of mice, circulating V-Ab complexes are present throughout life. In virtually all 9-month-old mice of the SWR/J strain persistently infected with LCM virus, complexes accumulate in heavy deposits in the glomeruli and are associated with severe nephritis. In contrast, mice of the same strain and age persistently infected with LDV had fewer glomerular deposits and clinical nephritis did not develop. Nearly 16 times more IgG was deposited in LCM

virus-infected kidneys (63.7 μg of Ig eluted per kidney) than in kidneys of LDV-infected mice (4.1 μg of Ig eluted per kidney) [OLDSTONE and DIXON, 1971a]. It is not known whether the difference between pathogenicity of LCM virus and LDV complexes is quantitative or related to peculiarities of the complexes formed.

Once complexes are formed and circulate, they can either be phagocytosed and removed by cells of the reticuloendothelial system (RES) or deposited in tissues. Large complexes appear to be preferentially removed by the RES over small complexes. Also, some cells of the RES have receptors which recognize different classes of Ig and complement and show different binding affinities. Changes in structure or recognition units of Ig seem important in phagocytosis, since alteration by reduction and alkylation of antibody (before making the soluble complex) results in a decreased rate of removal of complex from the circulation [MANNICK and AREND, 1971]. Depression of the RES occurs after prolonged exposure to circulating immune complexes. Decreased efficiency in RES function precedes the onset of immune complex deposits and subsequent development of proteinuria and nephritis. The phagocytic properties of mesangial cells of the kidney may then protect against deposits in the glomerular basement membrane, with such deposits occurring after mesangial cell overload [WILSON and DIXON, 1971].

Deposits of complexes in tissues is an active process. During deposition, increased vascular permeability occurs which is associated with release of vasoactive agents. With increase in vascular permeability, large complexes, usually 19S in size or larger, deposit along a filtering membrane. The sequence of immune complex deposition as studied in acute immune complex disease of rabbits [COCHRANE and KOFFLER, 1973] is as follows: (1) immune complex circulates in blood vessels; (2) in the presence of basophilic leukocytes with adherent IgE antibody, antigen induces release of a platelet-activating factor which then causes clumping and release of vasoactive amines; (3) vasoactive amines increase the permeability of blood vessels locally; and (4) macromolecular immune complexes enter and become trapped along filtering membranes to induce injury.

Studies have been done to determine the time required for the removal of complexes after their deposition in sites where injury takes place. Using the acute and chronic serum sickness model in rabbits for studying bovine serum albumin/antibovine serum albumin complexes. WILSON and DIXON [1971] found that the half-time disappearance of antigen in the renal glomeruli was 10.1 days in acute and 5.6 days in chronic disease.

2. Choroid Plexus of the Brain

Recently, evidence has indicated that the choroid plexus of the brain is also a favored site for deposition of immune complexes. This is not surprising, as the choroid plexus is anatomically similar to the renal glomerulus in that it consists of tufts of capillaries covered by a columnar epithelium (fig. 10). The endothelium, its basement membrane, a perivascular space and the tightly joined epithelium with its basement membrane constitute a barrier between the blood stream and the cerebrospinal fluid (fig. 10). In contrast to other vessels in the brain, the endothelium of capillaries in the choroid plexus is fenestrated. The endothelium of capillaries in the renal glomerulus is also fenestrated.

Persistent LCM viral infection of mice is the prototype experimental model for study of V-Ab immune complex disease [OLDSTONE and DIXON, 1971a, c]. In our laboratory histologic examinations of the choroid plexus from several strains of mice persistently infected with LCM virus since birth usually revealed no morphologic aberrations, although on occasion dense deposits surrounded by macrophages have been seen. Immunofluorescent study of the choroid plexus has indicated the presence of IgG, C3 and viral antigen occurring as irregular granular deposits (fig. 11). Such deposits occurred in 40% of mice by 4 months of age and nearly all mice by 9 months of age. Fluoresceinated antibodies to albumin and fibrinogen did not stain the choroid plexus, indicating that the deposits seen did not represent nonspecific trapping of serum proteins. Age-matched uninfected controls as well as 6- to 8-month-old mice chronically infected with scrapie agent and showing clinical signs of disease had no immune complex deposits in the choroid plexus. The scrapie agent causes a progressive noninflammatory spongiform encephalopathy; circulating infectious complexes have not been reported, nor have antibody deposits been demonstrated in the brains of animals with this disease [KATZ and KOPROWSKI, GIBBS and GAJDUSEK, personal commun.; LAMPERT *et al.*, 1972]. Electron microscopy of choroid plexus from those mice persistently infected with LCM virus revealed patchy, electron-dense material, predominantly within extracellular, perivascular spaces (fig. 12) but also within or adjacent to the epithelial or vascular basement membrane. Leukocytic infiltrates were apparent in the choroid plexus in some animals but there was no evidence of alterations of the epithelial or endothelial cells.

In view of the potentially pathogenic role of antigen-antibody complexes in the central nervous system, we also studied the choroid plexus from humans with systemic lupus erythematosus (SLE). SLE closely resembles experimental serum sickness; the tissue injury in SLE is mediated by antigen-

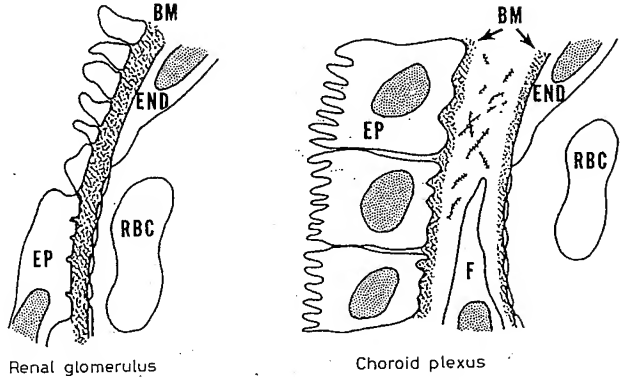

Fig. 10. Morphologic comparison between the renal glomerulus and choroid plexus. RBC, red blood cell; END, endothelial cell; BM, basement membrane; F, fibroblast; EP, epithelial cell.

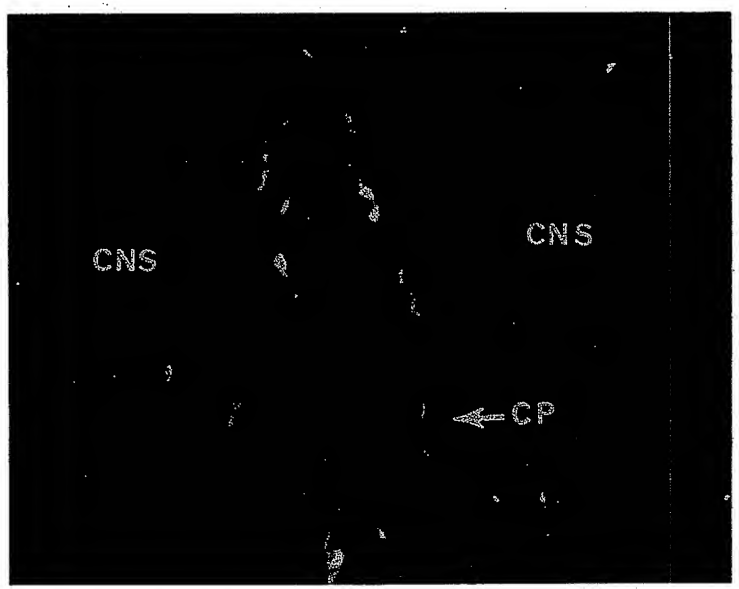

Fig. 11. Fluorescent photomicrograph showing IgG deposits in the choroid plexus of a 6-month-old SWR/J mouse chronically infected with LCM virus. A 4-μm thick frozen section was stained with fluoresceinated antiserum to mouse IgG. Note scattered IgG deposits. CNS, central nervous system parenchyma; CP, choroid plexus. Similar results were obtained with fluorescein conjugated antiserum to mouse C3 while fluorescein conjugated antisera to mouse albumin and fibrinogen failed to stain these deposits.

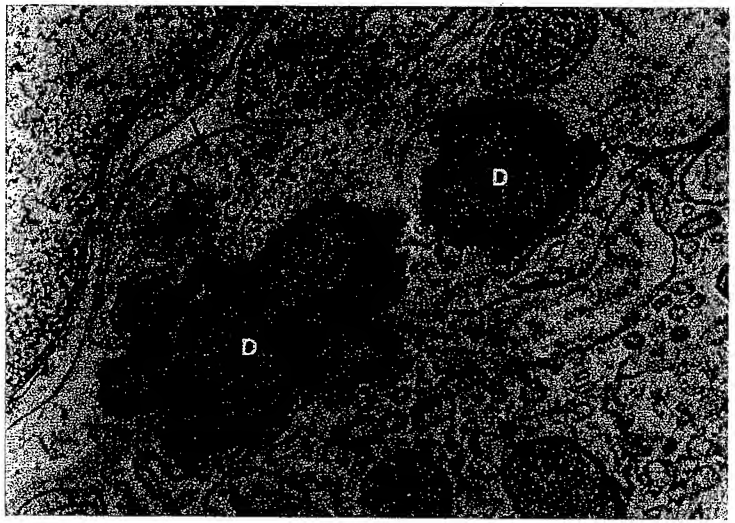

Fig. 12. Electron photomicrograph of large electron-dense deposits (D) in the perivascular space of the choroid plexus of a 9-month-old SWR/J mouse persistently infected with LCM virus. Note phagocytosis of a portion of the deposit by a macrophage (arrow).

antibody complexes, most notably antibody to DNA-DNA and antibody to nucleic antigen-nucleic antigen complexes [reviewed by COCHRANE and KOFFLER, 1973]. Nervous and mental disorders have been recorded in 75% of patients with SLE, but neuropathologic correlations are frequently difficult to establish [DUBOIS and TUFFANELLI, 1964; DIETZE and VOEGELE, 1966; JOHNSON and RICHARDSON, 1968]. In our examination of autopsy material from four patients with SLE who had neurologic disorders, we found all had high antibody titers to DNA and nuclear antigens, scattered granular deposits of IgG and C3 in the choroid plexus, and dense patchy deposits as revealed by fluorescent and electron microscopy. In addition, over 60 $(NZB \times W)F_1$ female mice, used as animal models of SLE, showed similar choroid plexus aberrations [LAMPERT and OLDSTONE, 1973].

These observations indicate that trapping of complexes in the choroid plexus can occur in natural or experimental immune complex disease. Immune complexes trapped in the choroid plexus may activate the complement system and release vasoactive factors. These substances could cause changes

in the permeability of membranes, resulting in shifts of water, electrolyte, and pH balance which might in turn result in diffuse or focal central nervous system disturbances. Indeed, among some patients with SLE who have neurologic or psychiatric disturbances, there is evidence that the complement system is activated, with resultant lowering of C4 hemolytic activity [PETZ *et al.*, 1971] and that DNA-antibody to DNA complexes occurs in the cerebrospinal fluid [HARBECK *et al.*, 1973]. It is an open question as to whether some of the nervous system and behavioral derangements accompanying acute and chronic viral infections may be caused by V-Ab immune complexes and whether some chronic behavioral disorders of unknown etiology in man may be associated with immune complex disease.

3. Blood Vessels

Immune complexes also deposit in blood vessels, specifically arteries, causing arteritis. In experimental acute immune complex disease the incidence of arteritis is greatest in the coronary outflow area at the points of branching of the aorta and in the pulmonary arteries [COCHRANE and KOFFLER, 1973], while in chronic virus infections in man and animals, arteritis is found scattered in various tissues throughout the body. Histologically, the arteritis begins with mild proliferation of the endothelial cell of the intima. Subsequently, neutrophils enter the reaction site, degrade the internal elastic lamina, enter the media and adventitia, and fibrinoid necrosis develops. Viral antigen, host Ig and C3 can be detected in the vessel wall by fluorescent microscopy (fig. 13, 14).

In several experimental or naturally occurring virus infections, V-Ab immune complexes deposit in vessels and cause vasculitis. The finding of such complexes in mink chronically infected with Aleutian disease virus [HENSON *et al.*, 1963; PORTER *et al.*, 1969, 1973], horses infected with equine arteritis virus [DOLL *et al.*, 1957] and mice chronically infected with lymphocytic choriomeningitis virus [OLDSTONE and DIXON, 1970b] suggests that vasculitis might also be found in association with virus infection in man. Convincing evidence of an association between HB Ag-positive infection, persistent antigen and antibody in the circulation, and polyarteritis nodosa has accumulated from several laboratories [GOCKE *et al.*, 1971; NOWOSLAWSKI *et al.*, 1972; EDGINGTON, personal commun.]. Recently, NOWOSLAWSKI and co-workers have shown the localization of HB Ag, Ig (presumably antibody to HB Ag) and complement in the vessel walls of several patients with polyarteritis who were not suspected of having viral hepatitis type B [NOWOSLAWSKI, in press].

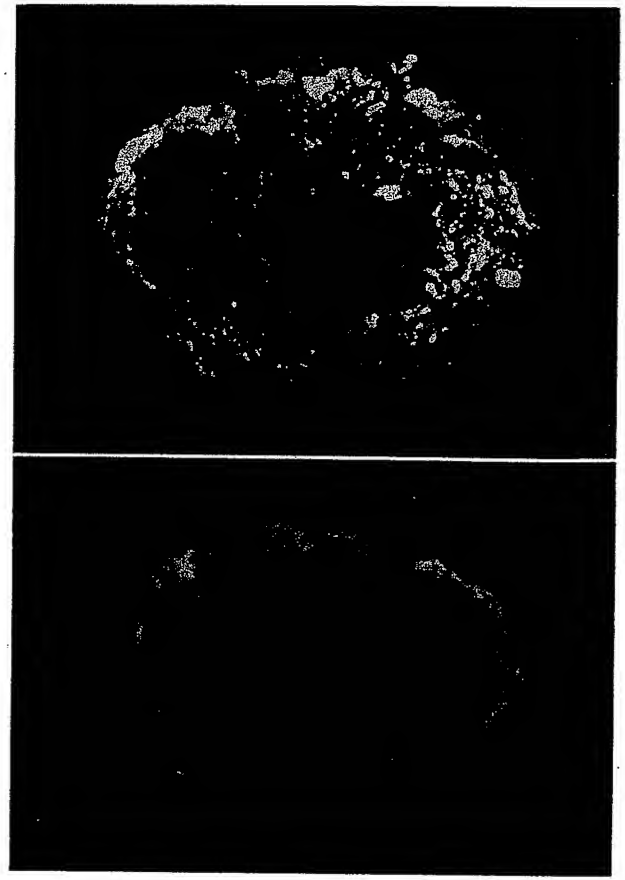

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Fig. 13. Fluorescent photomicrograph of an artery from a patient with chronic persistent hepatitis associated with HB Ag-positive infection. Tissue sections stained with fluoresceinated antiserum to human IgG. Note the granular and lumpy deposits of IgG. Similar results were obtained when consecutive sections were stained for deposits of host IgM, C3 and HB Ag. (Picture kindly supplied by Dr. A. NOWOSLAWSKI, Department of Immunopathology, State Institute of Hygiene, Warsaw, Poland.)

Fig. 14. Fluorescent photomicrograph of an artery from a patient with chronic persistent hepatitis associated with HB Ag-positive infection. Tissue section was stained with Rhodamine conjugated antiserum to HB Ag and shows the granular pattern of antigen deposited in the vessel wall. Observe the similarity between these deposits and the deposits of IgG seen in figure 12. (Picture courtesy of Dr. A. NOWOSLAWSKI, Department of Immunopathology, State Institute of Hygiene, Warsaw, Poland.)

4. Other Tissues

In experimental immune complex disease (acute or chronic serum sickness), injury due to deposits of immune complexes occurs in many tissues, including heart, lungs and joints [reviewed by COCHRANE and KOFFLER, 1973], while in patients with SLE, complexes are also found in the skin. This suggests that similar tissue injuries might be related to V-Ab complexes in man. In most acute virus infections the association of viremia with immune response, occurrence of rash, behavioral disturbances, myalgia and arthralgia is remarkably similar to the pattern seen in experimental serum sickness. V-Ab immune complexes are also found in the liver [NOWOSLAWSKI *et al.*, 1972; EDGINGTON, personal commun.] and placenta [OLDING and OLDSTONE, unpublished observations] and may account for part of the liver disease seen with HB Ag-positive infection in addition to being implicated in fetal and placental disorders.

Part of the liver injury associated with HB Ag-positive infection is probably due to antiviral antibody-complement mediated injury taking place on the surface of hepatic parenchymal cells. Immunofluorescent study of sections of liver from such patients shows deposition of host Ig, C3 and HB Ag on and in the injured cells [NOWOSLAWSKI *et al.*, 1972; EDGINGTON, personal commun.]. Antibody-mediated complement cytolysis occurs after the complexing of viral antigen(s), antiviral antibody and complement at a susceptible membrane site. The destruction of virus-infected cells through the interaction of antiviral antibody and complement has been reported for many viruses [OLDSTONE and DIXON, 1973], suggesting that antiviral antibody may play a role in the production of tissue injury in viral infections and that antiviral antibody-mediated cytolysis is a general biological phenomenon. It is not known whether the antibodies involved *in vivo* are themselves lytic for the infected cells or if the attachment of nonlytic antiviral antibody to the plasma membrane of infected cells can accelerate the killing of these cells by lymphocytes and activated macrophages.

D. Immune Complex Disease in Chronic Viral Infections

Immune complex disease occurs in most, if not all, virus infections in which there is both ongoing viral replication and a continuous host immune response (see table I for complete list). For example, in viral hepatitis type B in man, circulating virus-host Ig complexes have been found in the serum [EDGINGTON, personal commun.; ALMEIDA and WATERSON, 1969b] and

virus, host Ig and complement have been found deposited in a granular pattern in the renal glomeruli, arteries and liver (sect. III. C) [COMBES *et al.*, 1971; GOCKE *et al.*, 1971; NOWOSLAWSKI *et al.*, 1972; NOWOSLAWSKI, in press; EDGINGTON, personal commun.]. Further, the Ig has been eluted from such deposited complexes and identified as specific antibody to HB Ag [NOWOSLAWSKI *et al.*, 1972; NOWOSLAWSKI, in press].

Several investigators [DAYAN and STOKES, 1972; PHILLIPS, 1972; LAPOINTE *et al.*, 1972] have demonstrated Ig, measles antigen and C3 in the renal glomeruli in granular deposits, while others have noted Ig deposits in the vessels of patients with subacute sclerosing panencephalitis [WHITAKER and ENGEL, 1972]. Similar findings may be found in humans chronically infected with rubella virus and cytomegalovirus.

In patients with Burkitt's lymphoma, circulating Epstein-Barr virus (EBV)-antibody complexes [MAKOJIMA *et al.*, 1973] and deposited EBV, host Ig and C3 have been found in a granular pattern along the basement membrane of the renal glomeruli [OLDSTONE and KLEIN, unpublished observations]. MAKOJIMA *et al.* [1973] found that the serum from a patient with Burkitt's lymphoma contained antibodies to EBV as well as EBV antigens complexed together. We have examined kidney tissues from Burkitt patients in our laboratory and have found the characteristic pattern of immune complex deposits. Acid elution and identification of the deposited Ig from the renal glomeruli of these tissues indicated immunologic specificity with EBV antigens [OLDSTONE and KLEIN, unpublished observations]. This study shows that glomeruli are a fruitful source for isolation of virus or antiviral antibody and may be of use in uncovering the etiologic agent(s) in those diseases of unknown causes in which chronic infection is suspected. For example, in the spontaneous, naturally occurring leukemia of AKR mice it has been possible to demonstrate the presence of immune complex deposits in the renal glomeruli [OLDSTONE *et al.*, 1972b] and to recover specific antibodies to Gross cell surface antigen [OLDSTONE *et al.*, 1971] and antibodies to RNA-dependent DNA polymerase of murine leukemia virus [HOLLIS *et al.*, 1974]. Rodent and avian leukemias and lymphomas are caused by oncornaviruses. Whether similar diseases in man are also caused by persistent oncornavirus infection is under active investigation. Hence, all reports of Ig and C3 granular deposits in patients with leukemia, lymphoma and other cancers are of extreme interest [LEWIS *et al.*, 1971; SUTHERLAND and MARTINEZ, 1973; OLDSTONE and PARKS, unpublished observations]. SUTHERLAND and MARTINEZ [1973] have reported the occurrence of oncornavirus group-specific (gs) antigen to feline leukemia virus in the glomeruli of two patients

with leukemia and lymphoma. In our laboratory Ig eluted from kidneys of 7 patients with cancer failed, at the concentrations used, to show any detectable antibodies to gs antigen of wooley monkey or of murine leukemia viruses [OLDSTONE, PARKS and DIXON, unpublished observations].

IV. Conclusion

Virus-antibody-complement union affords a host protection in terms of neutralization and clearing of unwanted infectious agents; however, the same union also affords a host the hazard of virus-induced immune complex disease. In persistent, relatively noncytopathic viral infections, tissue injury is a direct result of both the host's antiviral immune response and the interaction of that immune response with virus or viral antigens. Factors affecting the amount of virus carried and the magnitude of the antiviral immune response are of prime importance in determining the accompanying tissue injury and disease processes.

Immunopathologic study indicates that immune complex disease occurs frequently in association with chronic viral infections and that it thus may be a pathogenic mechanism common to human nephritis and arteritis. The future definition of the mechanisms governing the production and deposition of immune complexes and the identification of the antigens and antibodies involved will undoubtedly further characterize a variety of human disorders of unknown origin.

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